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Ilelic Acids A and B, Two Unusual Triterpenes with a Seven-Membered Ring from *Ilex latifolia*

Cun-Qin Wang,[†,‡] Lei Wang,[*,‡,§] Chun-Lin Fan,[‡,§] Dong-Mei Zhang,[‡,§] Xiao-Jun Huang,[‡,§] Ren-Wang Jiang,[‡,§] Liang-Liang Bai,[‡,§] Jun-Min Shi,[‡,§] Ying Wang,[*,‡,§] and Wen-Cai Ye[*,†,‡,§]

Department of Phytochemistry, China Pharmaceutical University, Nanjing 210009, People's Republic of China, and Institute of Traditional Chinese Medicine & Natural Products and Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, College of Pharmacy, Jinan University, Guangzhou 510632, People's Republic of China

chywc@yahoo.com.cn; cpuwanglei@126.com; wangying_cpu@163.com

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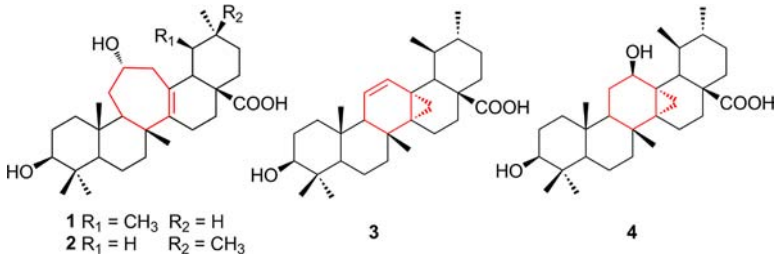
ABSTRACT



Two unusual triterpenes, ilelic acids A (1) and B (2), together with their biosynthetic related compounds ilelic acids C (3) and D (4) were isolated from the leaves of *Ilex latifolia*. Their structures with absolute configurations were elucidated by spectroscopic analysis and modified Mosher's method. The plausible biogenetic pathway of 1 and 2 is proposed. These triterpenes exhibited a potent inhibitory effect on MCF-7 and MDA-MB-231 cells.

The leaves of *Ilex latifolia* (Aquifoliaceae) have long been used as an herbal tea (Ku-Ding-Cha) in China for adjuvant treatment of cold, headache, hypertension, and diabetes.[1] Previous phytochemical studies of this plant had led to the isolation of more than 60 triterpenes and triterpenoid saponins,[2,3] which demonstrated antidepressant,[4] antibacterial,[5] and antitumor activities.[6] As part of a program to search for structurally unique and biologically interesting natural products,[7,8] ilelic acid A (**1**), which represented a new type of triterpenoid with a seven-membered ring, named as "Ilesane", and a rare dubosane-type triterpenoid,[9] ilelic acid B (**2**), were isolated from the leaves of *I. latifolia*. In addition, the biosynthetic related compounds including three hexacyclic triterpenoids (**3**–**5**) and two C-27 oxygenated triterpenoids (**6**–**7**) were obtained

[†] China Pharmaceutical University.
[‡] Institute of Traditional Chinese Medicine & Natural Products, Jinan University.
[§] Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, Jinan University.

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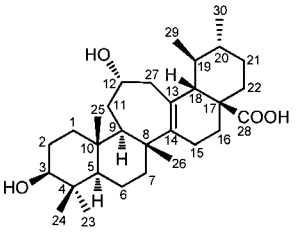
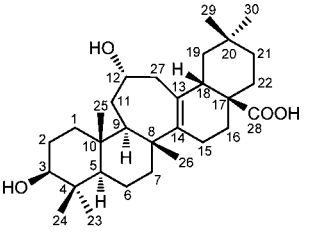
from the title plant.[10] Herein, we report the isolation and structure elucidation of **1** and **2**. The plausible biogenetic pathway of **1** and **2** and the antitumor activities of these triterpenoids are also described.



1



2

The air-dried leaves of *I. latifolia* were pulverized and extracted with water under reflux. The water extract was subjected to a D101 macroporous resin column eluted successively with H_2O, 45%−95% EtOH. The fraction eluted by 75% EtOH was purified by chromatography on silica gel, reversed-phase C_{18} silica gel, and preparative HPLC to yield compounds **1**−**9**.

Ilelic acid A (**1**) was obtained as amorphous powder. The molecular formula of **1** was determined as $C_{30}H_{48}O_4$ on the basis of a quasi-molecular ion at m/z 495.3444 $[M+Na]^+$ (calcd 495.3444 for $C_{30}H_{48}O_4Na$) in the HR-ESI-MS. The IR spectrum showed the characteristic absorptions attributable to carboxyl (1708 cm^{-1}) and hydroxyl (3410 cm^{-1}) groups. The analysis of ^1H and ^{13}C NMR spectra revealed that **1** possessed 30 carbons, including four tertiary methyls [δ_H 1.21, 1.03, 0.94, and 1.12 (each 3H, s); δ_C 28.8, 17.0, 16.6, and 19.7], two secondary methyls [δ_H 1.01 (3H, d, J = 6.5 Hz) and 0.91 (3H, d, J = 6.0 Hz); δ_C 18.0 and 20.3], two oxygenated methines [δ_H 3.40 and 4.39 (each 1H); δ_C 77.8 and 69.4], and two olefinic carbons [δ_C 131.7 and 142.5] as well as one carbonyl carbon [δ_C 180.6]. The above spectral data suggested **1** could be a pentacyclic triterpenoid. With the aid of $^1H-^1H$ COSY, HSQC, HMBC, and ROESY experiments, the ^1H and ^{13}C NMR signals of **1** were assigned as shown in Table 1.

The ^1H and ^{13}C NMR signals assigned to rings A, B, and E (Figure 1) were similar to those of ursolic acid (**8**)[11] (Scheme 1). The presence of a methene and the absence of an angular methyl in comparison with the usual ursanes[11,12] suggested that **1** should possess six methyls and a seven-membered ring C, which was similar to the skeleton of duboscic acid.[9] The $^1H-^1H$ COSY spectrum revealed the presence of the spin-coupling systems in bold as shown in Figure 1. The HMBC correlations between H-27 (δ_H 2.66, 3.24) and C-11 (δ_C 35.1), C-13 (δ_C 131.7), C-14 (δ_C 142.5), and C-18 (δ_C 58.4) confirmed that C-27

(10) New compounds **3**, **4** and known compounds **5**–**9** were also isolated from *I. latifolia*. Their structures were elucidated by spectroscopic analysis and single-crystal X-ray diffraction (see Supporting Information).

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Table 1. NMR Data of **1** and **2** (in C_5D_5N, J in Hz)[a]

	1		**2**	
no.	δ_H	δ_C	δ_H	δ_C
1	α 1.14	40.1	α 1.20	40.2
	β 1.92		β 1.96	
2	α 1.82	28.4	α 1.82	28.3
	β 1.82		β 1.82	
3	3.40 (dd, 8.8, 7.2)	77.8	3.45 (dd, 9.0, 6.9)	77.9
4	–	39.3	–	39.4
5	0.90	55.4	0.92	55.3
6	α 1.34	19.4	α 1.36	19.5
	β 1.55		β 1.58	
7	α 1.33	42.3	α 1.34	42.1
	β 2.01		β 2.06	
8	–	43.8	–	44.4
9	1.94	52.2	2.03	51.3
10	–	38.6	–	38.6
11	α 1.93	35.1	α 1.93	34.2
	β 2.24		β 2.18	
12	4.39 (m)	69.4	4.52 (m)	69.1
13	–	131.7	–	130.2
14	–	142.5	–	139.1
15	α 2.15	22.8	α 2.31	23.5
	β 2.45		β 2.38	
16	α 1.94	24.2	α 1.99	25.7
	β 2.19		β 1.99	
17	–	49.9	–	45.5
18	2.48 (d, 11.2)	58.4	2.95 (dd, 10.2, 3.3)	45.2
19	1.22	39.0	α 1.32	41.7
			β 1.67	
20	1.06	38.7	–	30.7
21	α 1.48	31.1	α 1.26	34.8
	β 1.48		β 1.43	
22	α 1.82	34.8	α 1.80	30.7
	β 2.04		β 2.05	
23	1.21 (s)	28.8	1.24 (s)	28.8
24	1.03 (s)	17.0	1.03 (s)	16.5
25	0.94 (s)	16.6	0.95 (s)	17.5
26	1.12 (s)	19.7	1.09 (s)	20.4
27	α 3.24	49.1	α 3.23	44.5
	β 2.66		β 2.40	
28	–	180.6	–	180.3
29	1.01 (d, 6.5)	18.0	0.91 (s)	25.8
30	0.91 (d, 6.0)	20.2	0.94 (s)	31.6

[a] Overlapped signals were reported without designating multiplicity.

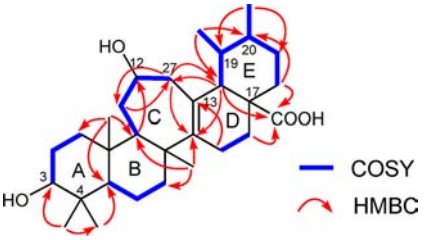


Figure 1. Key $^1H-^1H$ COSY and HMBC correlations of **1**.

was inserted between C-12 and C-13 to form a seven-membered ring C. The detailed interpretation of HMBC correlations (Figure 1) allowed the establishment of the planar structure of **1**.

In order to determine the relative configuration of **1**, the ROESY spectrum was extensively analyzed. The ROESY correlations between H-1α and H-5, between H-5 and H-9, between Me-25 and Me-26, and between H-18 and Me-29 indicated that **1** possessed the same A/B *trans*, B/C *trans*, and D/E *cis* ring junctions as usual ursanes (Figure 2). In addition, the ROESY correlations between H-3 and H-5, between H-12 and Me-26, and between H-12 and H-27β suggested the presence of 3β-OH and 12α-OH (Figure 2). Subsequently, the modified Mosher's method was applied to determine the absolute configuration of **1**.[13,14] Comparison of the ^{1}H NMR chemical shifts between (*S*)- and (*R*)-MTPA diesters of **1** (Figure 3) led to the assignment of *S* configurations of C-3 and C-12, respectively. Thus, the configurations of **1** were assigned as 3*S*, 5*R*, 8*R*, 9*R*, 10*R*, 12*S*, 17*S*, 18*S*, 19*S*, and 20*R*, respectively. Ilelic acid A (**1**) represents a new class of pentacyclic triterpenoid derived from ursolic acid. The name "Ilesane" was proposed for this type of triterpenoid.

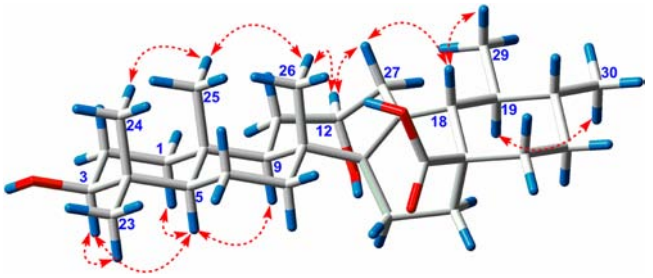
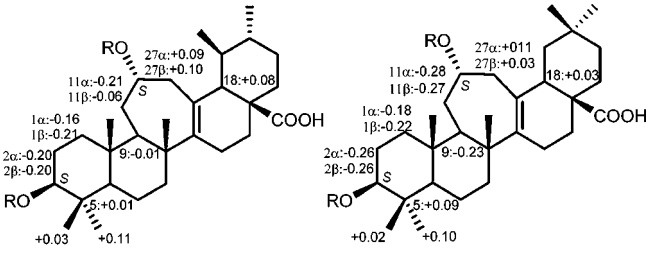


Figure 2. Key ROESY correlations of **1**.



1a: R = (*R*)-MTPA
1b: R = (*S*)-MTPA

2a: R = (*R*)-MTPA
2b: R = (*S*)-MTPA

Figure 3. $\Delta\delta$ values ($\Delta S - \Delta R$) for the MTPA diesters of **1** and **2**.

Ilelic acid B (**2**) showed the same molecular formula as **1** by its HR-ESI-MS (*m*/*z* 495.3448 $[M+Na]^+$; calcd for $C_{30}H_{48}O_4Na$: 495.3444). The IR spectrum of **2** was also similar to that of **1**. The ^{1}H NMR spectrum of **2** exhibited signals for six tertiary methyls at δ_H 1.24, 1.03, 0.95, 1.09, 0.91, and 0.94 (each 3H, s), as well as two oxygenated methine protons at δ_H 3.45 (1H, dd, J = 9.0, 6.9 Hz) and 4.52 (1H, m). The ^{13}C NMR and DEPT spectra displayed 30 signals for 6 methyls, 11 methylenes, 5 methines, and 8 quaternary carbons. Comparison of the NMR data of **2** with those of **1** (Table 1) revealed that most of signals were similar, except for the signals due to ring E. In the HMBC spectrum of **2**, the correlations between Me-29 (δ_H 0.91)/Me-30 (δ_H 0.94) and C-19 (δ_C 41.7), C-20 (δ_C 30.7), and C-21 (δ_C 34.8) were observed, indicating that Me-29 and Me-30 were both connected to C-20. A comprehensive analysis of the ^{1}H−^{1}H COSY, HSQC, HMBC, and ROESY spectra allowed the assignment of NMR data of **2** as shown in Table 1. The carbon skeleton of **2** belonged to a unique dubosane-type triterpene. The first example of this skeleton, duboscic acid, had been recently reported with a relative configuration.[9] As in the case of **1**, the result of the modified Mosher's method suggested that the absolute configurations of C-3 and C-12 of **2** were both *S* (Figure 3). Therefore, the structure of **2** was established, and the absolute configurations were assigned as 3*S*, 5*R*, 8*R*, 9*R*, 10*R*, 12*S*, 17*R*, and 18*S*, respectively.

Oleanolic acid and ursolic acid are the most common triterpenes occurring in natural plant populations. Based on the plausible biogenetic pathway of duboscic acid,[9] **2** could be derived from oleanolic acid (**9**) by oxygenation and migration of C-27 methyl, forming a seven-membered ring C. Similarly, ilelic acid A (**1**) could be derived from ursolic acid (**8**) by the same procedure (Scheme 1). It is noteworthy that the key intermediates **6**−**7**, which could easily form the C-27 carbocations, and several biosynthetic related hexacyclic triterpenoids (**3**−**5**) were also found in the same plant,[10] rationalized the proposed biogenetic pathway. In addition, compound **4** could be formed by β-attack of the incoming nucleophile, in which the partial bond between C-12 and C-27 was broken under kinetic control, while compounds **1** and **2** should be produced by α-attack of the nucleophile with breaking of the partial bond between C-12 and C-13 under thermodynamic control.[15]

The growth inhibitory activities of these triterpenoids (**1**−**4**, **6**, and **7**) were evaluated in human breast cancer cells MCF-7 (estrogen receptor-positive) and MDA-MB-231 (estrogen receptor-negative). Compounds **1** and **2** showed a growth inhibitory effect against MCF-7 cells with IC_{50} values of 29.51 ± 3.44 and 38.49 ± 3.16 μM, respectively. Compound **7** was the most potent among all tested compounds with IC_{50} values of 12.65 ± 0.94 and 4.58 ± 0.56 μM, respectively, indicating that the *p*-(*E*)-coumaroyl moiety at the C-27 position may contribute to improving the growth inhibitory potential, when compared with the triterpene with the *p*-(*Z*)-coumaroyl moiety (**6**) (see Supporting

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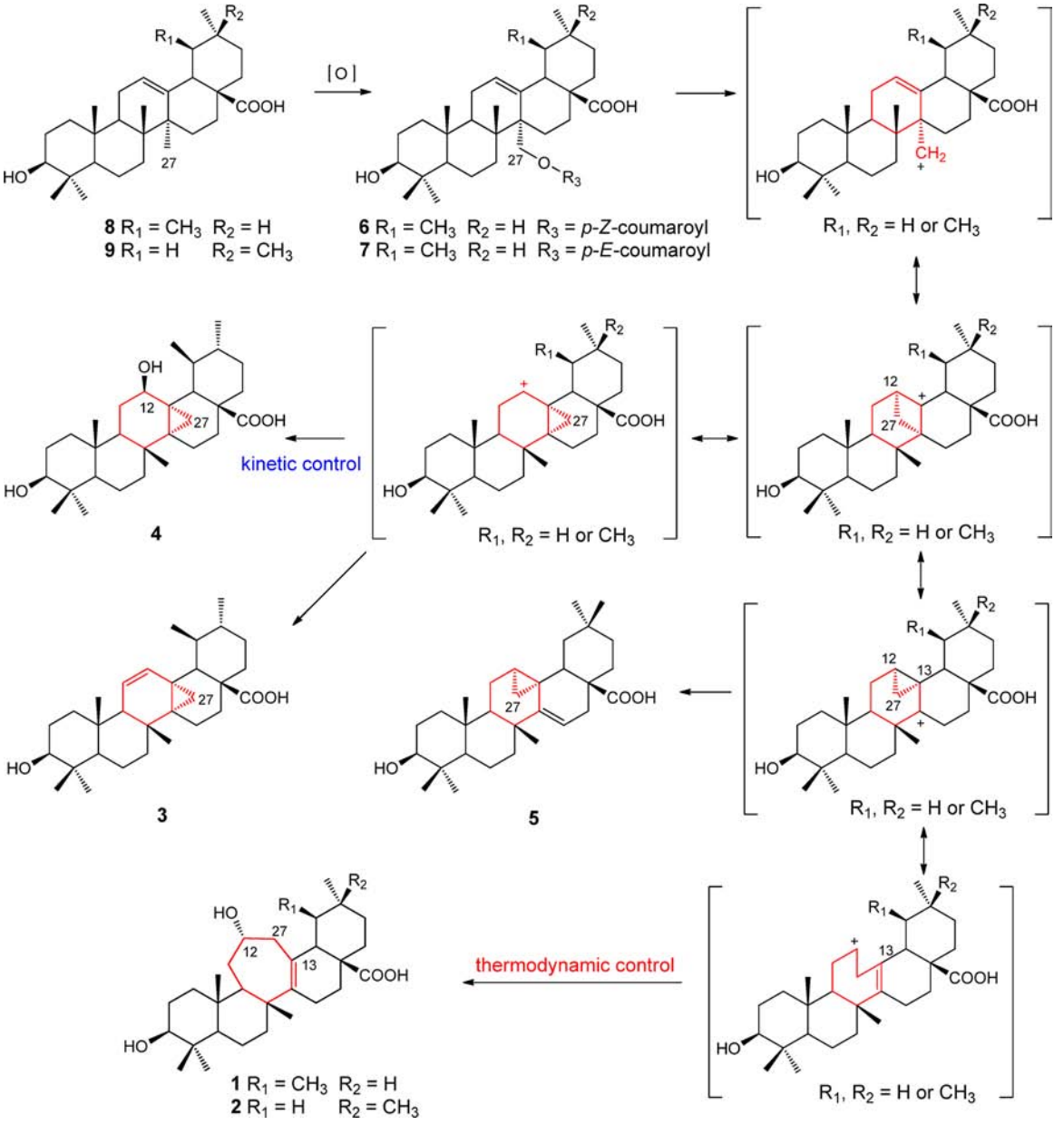
Scheme 1. Plausible Biogenetic Route of **1** and **2**



Information). In addition, all the tested triterpenoids showed more potent growth inhibitory activity in MCF-7 cells than in MDA-MB-231 cells, implying that their growth inhibitory activities may be partly dependent on the status of the estrogen receptor. It is necessary to further study the role of estrogen receptor and the molecular mechanism underlying the cell death induced by these triterpenoids.

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Supporting Information Available. Detailed description of the experimental procedure; a listing of UV, IR, HR-ESI-MS, HR-EI-MS, NMR spectral data of compounds **1**–**9**; X-ray data for **4** (CIF); NMR data of MTPA diesters of **1** and **2**; and bioassay data. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.